TITLE PAGE:

PEST MANAGEMENT GRANTS FINAL REPORT: 2001-2002
Contract Number: 00-0193S

Contract Title:	**Mass Release of Natural Enemies of Vine Mealybug**
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Contractor Organization:	**Foothill Agricultural Research (FAR)**
Date:	**February 2002**

Prepared for California Department of Pesticide Regulation

Disclaimer

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Acknowledgements

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This report was submitted in fulfillment of DPR contact number 00-0193S, Mass Release of Natural Enemies of Vine Mealybug, by Foothill Agricultural Research (FAR), under the partial sponsorship of the California Department of Pesticide Regulation. Work reported here was completed as of February 2002.

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TABLE OF CONTENTS

LIST OF TABLES:

LIST OF FIGURES:

ABSTRACT:

This is a final report for the contract term 2001-2002, on an effort by Coachella Valley table grape growers, a commercial insectary, University of California, Riverside (UCR) researchers and extension personnel to implement a long-term reduced-risk pest management system to control recently introduced vine mealybug (VMB) pests and promoting this approach to all growers and interested parties.

Three main objectives include: (**1**) establishing an IPM Innovator Program, (**2**) producing and releasing 2 parasites, *Anagyrus pseudococcae* and *Leptomastidea abnormis*, and (**3**) evaluating impact of parasites in reducing damage by VMB (including success in colonization and/or augmentation), and evaluating the impact of ant activity on parasite effectiveness. Many other growers and organizations are interested in this study, and are eagerly awaiting the final results.

Significant achievements for our three objectives include: (**1**) Collaborators have provided all resources needed to establish and operate viable study/demonstration sites. Outside interested parties are participating in the project's periodic meetings. (**2**) Insectary parasite production techniques have been modified, resulting in up to a 25 times increase in numbers of parasites produced from previous years, with a significant reduction in contamination of rearing colonies. The increase in production of parasites greatly enhances the potential to control VMB with biological control colonization and/or augmentation programs. (**3**) **a.** Releases of *Anagyrus* combined with ant control provided the most effective reduction in numbers of VMB in the spring, particularly on vines, where honeydew is produced and causes damage to the fruit, prior to harvest. **b.** Numbers of VMB were much higher than in previous years. Under these higher numbers of VMB, ant control by itself was not effective in reducing VMB. **c**. In three of four fields, release of both parasites plus ant control, was less effective than the separate release of each parasite by itself, plus ant control. In plots where both parasites were released, however, only one-half the numbers of each parasites were released, compared with the higher number of each parasite where they were released separately. **d.** Damage to fruit (= boxes washed to remove honeydew) was significantly lower in fields where parasites were released in previous years, compared with sites where parasites were not released in previous years. **e.** In our early harvest variety (Perlette) there was no damage to fruit despite very high numbers of VMB.

In summary, the accomplishments listed above provide a strong basis for an effective IPM program for VMB that begins in the spring of each year. At this time of year the VMB parasites are at maximum effectiveness.

BODY OF REPORT

A. INTRODUCTION

OBJECTIVES

1. Establish IPM Innovator Program
2. Rearing and releasing two species of VMB parasites.
3. Evaluation of the parasites' viability in plots; impact of parasite releases on VMB damage, and on yields at harvest; and, impact of ants on parasite effectiveness against VMB.

Objective 1

a. This objective has been accomplished. The team is in place and operational. In March 2000 we had a presentation of research and future plans to all growers in Coachella Valley at the Annual Meeting in Coachella of the California Desert Grape Growers. Growers approved funding for one more year based on our results. In the year 2001 meetings were held January 25th (planning session), November 5th and November 28th.

b. No changes

c. No problems

Objective 2

a. F.A.R. continues to fine tune mass rearing procedures of the two vine mealybug parasites, *Anagyrus pseudococci* and *Leptomastidea abnormis.* We have fulfilled this objective and within the budget.

b. Rearing of parasites is taking place at Foothill Ag Research, Inc., and release of parasites is done by their personnel in the Coachella Valley test plots. Rearing is being done on banana and butternut squash. F.A.R. will continue to experiment with other substrates for rearing VMB. Beginning in January 2000, parasites of both species were usually released once a week in all 4 farms (appendix table 1). Releases will continue through June 2002.

c. At the end of the regular growing season (after harvest) *Leptomastidea* were no longer released. *Anagyrus* were released at 3 levels to obtain preliminary data in anticipation of next years trials.

d. No problems

Objective 3

a. Funding for this program from DPR has been requested only to support objective 2. However, we include here a report on objective 3 because the results are promising, and they clearly demonstrate and justify the effort and funding provided for objective 2. Funds to support activities reported here under objective 3 are from other grant agencies. No funds from DPR were used to support the activities listed under objective 3.

The timetable is proceeding according to schedule. We have very conclusive data from the third year that supports our expectations that parasite releases combined with ant control can substantially reduce damage from VMB.

b. Evaluation is conducted by University of California personnel. The experimental design for year 2001 was a randomized complete block with five treatments each in four replications (four farms, see Fig 1). Each of four growers (members of the Coachella Valley IPM Innovator Group) provided approximately eight acres that were not treated with chemicals (except for ant control through skirt treatments) for five treatments: (a) Anagyrus release plus ant control, (b) *Leptomastidea* release plus ant control, (c) untreated (no an control, no parasite release), (d) ant control (no parasite release), (e) both parasites released plus ant control. Samples are taken only from the center third of each plot. The outer 1/3 on each side of the plots served as buffer zones between treatments. Plots were located on the up-wind edge of all farms not adjacent to other vineyards. This minimized insecticide drift, which kills parasites and predators.

Baseline Data: Data were collected for one week in each of the four vineyards. Pre-treatment ant control counts were also taken.

Chemical treatments (skirt applications) against ants were applied in one of the two parasite release plots and in one of the two untreated (except for ants) control plots (Fig 1). We used a registered material, Lorsban, for ant control and applying it with a modified sprayer we designed to minimize impact against parasites and predators.

Evaluation of impact from treatments on mealybugs and yields were based on sampling techniques developed over the past three years by D. Gonzalez, the late H. Shorey (Univ. Calif.), J. Ball, and K. Godfrey (CDFA). Evaluation samples were taken every two weeks at each farm by D. Gonzalez, a technician from UCR, and two field assistants. Samples were staggered allowing sampling of two farms on odd-numbered weeks and two farms on even-numbered weeks. Evaluations were based on the following:

Parasite numbers were assessed every two weeks on each farm beginning one month after the first release in March through November by placing 18 yellow sticky traps through the center third of the plots where parasites were released. Traps were left in the field for two weeks, and returned to the lab for identification and counts of parasites, predators, and mealybugs. Data from our trials in 1998 and 1999 showed these traps were as reliable as two other methods tested. Yellow traps have a great advantage in requiring a relatively short processing time thus allowing more samples to be collected. Similar samples are taken from the untreated control plots and from the commercial treatment plots. Pre-treatment samples were taken for one week to assess native parasite and predator activity in all plots prior to application of any treatments or parasite releases.

Damage from mealybugs was estimated with visual observations in time-controlled samples from March until harvest date. The relatively short time needed to take each sample allowed a greater number of samples with equal or greater sampling efficiency than other methods tested by D. Gonzalez, H. Shorey, J. Ball and K. Godfrey in 1998 and by D. González in 1999 and 2000. We had four samplers taking a total of 18 samples per each of five treatments every two weeks. Data recorded included numbers of ants, and mealybugs, listing sows and nymphs for mealybugs.

Estimates for ant abundance were taken from pitfall traps and additional visual observations. The pitfall traps sampled ants on the ground, and the visual counts sampled ants in the vines. There were 18 traps per plot. Eighteen vines were counted for number of ants observed in thirty seconds for each sample. Samples and visual observations were taken bimonthly.

Yields were recorded in boxes/acre (18-lb. equivalents) from each of the five treatment plots. In all treatments, we recorded yield from both fruit-washed and unwashed for honeydew removal. Fruit wash was done directly in the field by dipping fruit with honeydew into 5-gal buckets of water and setting them aside to dry. These were packed into separate boxes for recording boxes/acre of washed fruit.

c. Changes at the end of the growing season are the same as those described above under Obective 2, item 2c.

B. RESULTS

Objective 1.

We held 3 meetings in 2001: 25 January, 5 and 28 November. The January meeting was a planning session with our collaborators. The 5 November meeting was a progress report and planning session with our collaborators. The 28 November meeting was an annual report to the California Desert Grape administrative Committee at their annual meeting.

We have promoted the project and its results by contacting other interested parties through trade publications, grower organizations, the Riverside County Agricultural Commissioner's Office, and University of California Cooperative Extension personnel.

Objective 2

The *Anagyrus* will host feed on 1st instars and lay eggs on 2nd instars through adults. However, they produce mostly males, as much as 80%, on the 2nd and 3rd instars. On the 4th and 5th instars mostly females are produced, 60 to 70%.
The *Leptomastidea* lays eggs on the 1st instar producing 80% males. On the 2nd and 3rd instars they produce 60 to 70% females. They do not attack the 4th and 5th instars of the mealybug.

Crawler production, the most vital part of the culture, is extremely sensitive to microclimates and the quality of the squash. Slight changes in the temperature or humidity can cause severe crawler reduction. Our thermostats for cooling and heating are not accurate enough to maintain a constant temperature of 80ºF. During the summer months we have found that the females mature much earlier. To compensate for this occurrence the rotation of the crawler racks was reduced from every six weeks to every three weeks.

Also, the enclosed mealybug production cabinets are now thoroughly cleaned every two months. This is to further reduce the possibility of contamination. The open shelves are used for a six to eight week period while the cabinets are cleaned and new production is begun.

The mealybug used as the host for the two parasites is now being based on the size or instar, not the numbers of days of infestation. The size of the mealybug can be affected by the temperature. Therefore, during hot weather the mealybug may grow faster than during cold weather. However, as a general guideline we use 10-15 day old mealybug for *Leptomastidea* and 18-28 day old mealybug for *Anagyrus.*

Objective 3

A summary of our results follows: (i) VMB were reduced to the lowest level by *Anagyrus* releases plus ant control, compared with all other treatments on all fields (Fig 2) VMB numbers were also significantly lower in fields where parasites were released over two years compared with a field where parasites were only released for one year, SWI vs SWII, Fig. 2; (**ii**) ant numbers (Fig. 3) were not consistently related to numbers of VMB (Fig 2) as they have been in past years. Treatments were not effective in reducing ant numbers in three of four ranches. The cause of this is unknown but could be that higher numbers of VMB resulted in more food for the ants; (**iii**) numbers of parasites collected on sticky traps (Figs.4,5) showed that both species were recovered in greatest numbers at the end of the season when numbers of VMB were at their highest levels. More *Leptomastidea* were collected from the three farms where releases were made in previous years (SWI, Tudor, Bianco) compared with SWII where no releases were made before this year suggesting some survival during the winter. More *Leptomastidea* were recovered from release than from non-release plots. Higher numbers of *Leptomastidea* were collected on the west end of the valley (SWI and II, Bianco release sites) than at Tudor on the east side of the valley. *Anagyrus* were more abundant from release sites at Tudor on the east than at SW and Bianco on the west. *Anagyrus* were recovered in greater numbers in release versus non-release plots. Samples were taken at the end of the season to obtain preliminary data on percent parasitization by the two parasite species released, from different treatments (Fig. 6) in different fields (Fig. 7). From these results it is very clear that at the end of the grape growing season, just prior to harvest, an overwhelming majority of parasites were *Anagyrus*, with very few *Leptomastidea*; (**iv**) damage to fruit (from honeydew production by VMB), (Fig. 6) remains a complex interaction among numbers of VMB at the end of the season prior to harvest, time of harvest (determined in part by spring temperatures but mostly by the variety), and duration of parasite releases. Within the same variety less damage occurred where parasites had been released for two years (SW I, Fig 6) with more damage where releases were made only this year (SW II, Fig. 6). (**v**) despite great differences in numbers of VMB among treatments (Fig. 2), yields (Fig. 7) do not appear to be directly influenced by VMB numbers, especially in early harvest varieties such as Perlette at the Bianco Ranch.

Based on results in the spring of 2001 we have selected *Anagyrus* for further evaluation regarding effective numbers to release. Beginning 10 June we have been releasing *Anagyrus* at three densities and monitoring their impact against VMB (Fig. 10).

In results from these preliminary trials, we find that 18,000 *Anagyrus*/week/acre provides effective VMB reductions whereas 9,000 *Anagyrus*/week/acre does not.

C. DISCUSSION

It is clear from our results that in the Coachella Valley, *Anagyrus* releases were far more effective than those of *Leptomastidea* in reducing VMB to tolerable levels. From the results over three years we believe tolerable levels of VMB to be less than approximately 50 adult or late instar VMB on vines for a period not to exceeding three weeks. These are tentative suggestions which we will examine further in our 2002 trials.

Growers are interested in our results but they are emphatic that parasite release must be cost-competitive with insecticides. Our trials in 2002 will attempt to address this concern

SUMMARY AND CONCLUSIONS

The results over the past three years clearly support our hypothesis that releases of *Anagyrus* plus ant control can effectively reduce VMB to tolerable levels.

We need to define more clearly the minimum number of parasites to release, the optimal time seasonally) for the releases, and the minimum number of releases. We also need more effective ant control. That is, preferably a bait that can be used closer to harvest than the currently available materials for ant control.

We will resolve many of these questions in our trials in 2002.

APPENDICES

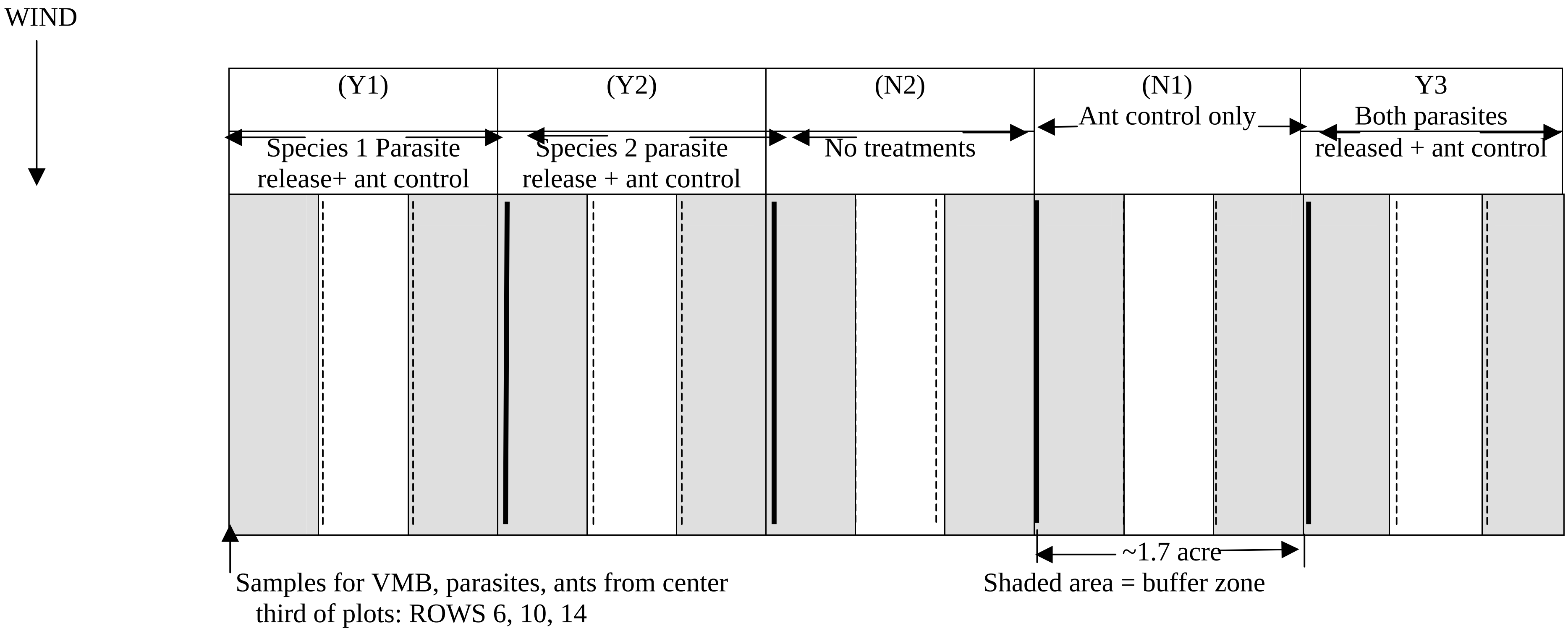
10 Figures and 1 Table

Table 1.

F.A.R., INC.
VINE MEALYBUG PROJECT
PARASITE PRODUCTION & RELEASES
JANUARY, 2001 - NOVEMBER, 2001

DATE	ANAGYRUS PRODUCTION	ANAGYRUS RELEASES	LEPTOMASTIDEA PRODUCTION	LEPTOMASTIDEA RELEASES
WEEK 1/1/01	451,000		220,000	
WEEK 1/7/01	610,000		260,000	
WEEK 1/14/01	820,000		270,000	
WEEK 1/21/01	720,000		365,000	
WEEK 1/28/01	692,000		150,000	
WEEK 2/4/01	768,000		140,000	
WEEK 2/11/01	583,000		135,000	
WEEK 2/18/01	676,000		145,000	
WEEK 2/25/01	836,000		231,000	
WEEK 3/4/01	1,297,000		252,000	
WEEK 3/11/01	1,045,000		180,000	
WEEK 3/18/01	653,000		218,000	
WEEK 3/25/01	820,000		343,000	
WEEK 4/1/01	900,000	50,000	334,000	50,000
WEEK 4/8/01	827,000	60,000	225,000	60,000
WEEK 4/15/01	766,000	48,000	153,000	48,000
WEEK 4/22/01	895,000	108,000	244,000	108,000
WEEK 4/29/01	858,000	216,000	513,000	216,000
WEEK 5/6/01	687,000	216,000	521,000	216,000
WEEK 5/13/01	616,000	108,000	388,000	108,000
WEEK 5/20/01	408,000	108,000	388,000	108,000
WEEK 5/27/01	429,000	108,000	358,000	108,000
WEEK 6/3/01	288,000	0	0	0
WEEK 6/10/01	328,000	108,000	0	0
WEEK 6/17/01	359,000	144,000	0	0
WEEK 6/24/01	476,000	144,000	0	0
WEEK 7/1/01	520,000	144,000	0	0
WEEK 7/8/01	614,000	144,000	0	0
WEEK 7/15/01	414,000	144,000	0	0
WEEK 7/22/01	335,000	0	0	0
WEEK 7/29/01	605,000	144,000	0	0
WEEK 8/5/01	519,000	144,000	0	0
WEEK 8/12/01	539,000	144,000	0	0
WEEK 8/19/01	464,000	144,000	0	0
WEEK 8/26/01	469,000	144,000	0	0
WEEK 9/2/01	449,000	144,000	0	0
WEEK 9/9/01	439,000	144,000	0	0
WEEK 9/16/01	576,000	216,000	0	0
WEEK 9/23/01	501,000	216,000	0	0
WEEK 9/30/01	481,000	216,000	0	0
WEEK 10/7/01	501,000	216,000	0	0
WEEK 10/14/01	298,000	108,000	0	0
WEEK 10/21/01	363,000	108,000	0	0
WEEK 10/28/01	303,000	108,000	0	0
WEEK 11/04/01	358,000	108,000	0	0
WEEK 11/11/01	343,000	108,000	0	0
WEEK 11/18/01	195,000	HOLIDAY	0	0
TOTAL	27,094,000	4,262,000	6,033,000	1,022,000

Fig. 1. Schematic Diagram of Experimental Design (field-plot arrangement)



No insecticide applications up-wind

HARVEST DATA: 20 rows from each plot

Y = parasite release
N = no parasite release

Figure 2. Mean Number of VMB on Vines from 4 Ranches, 2001.

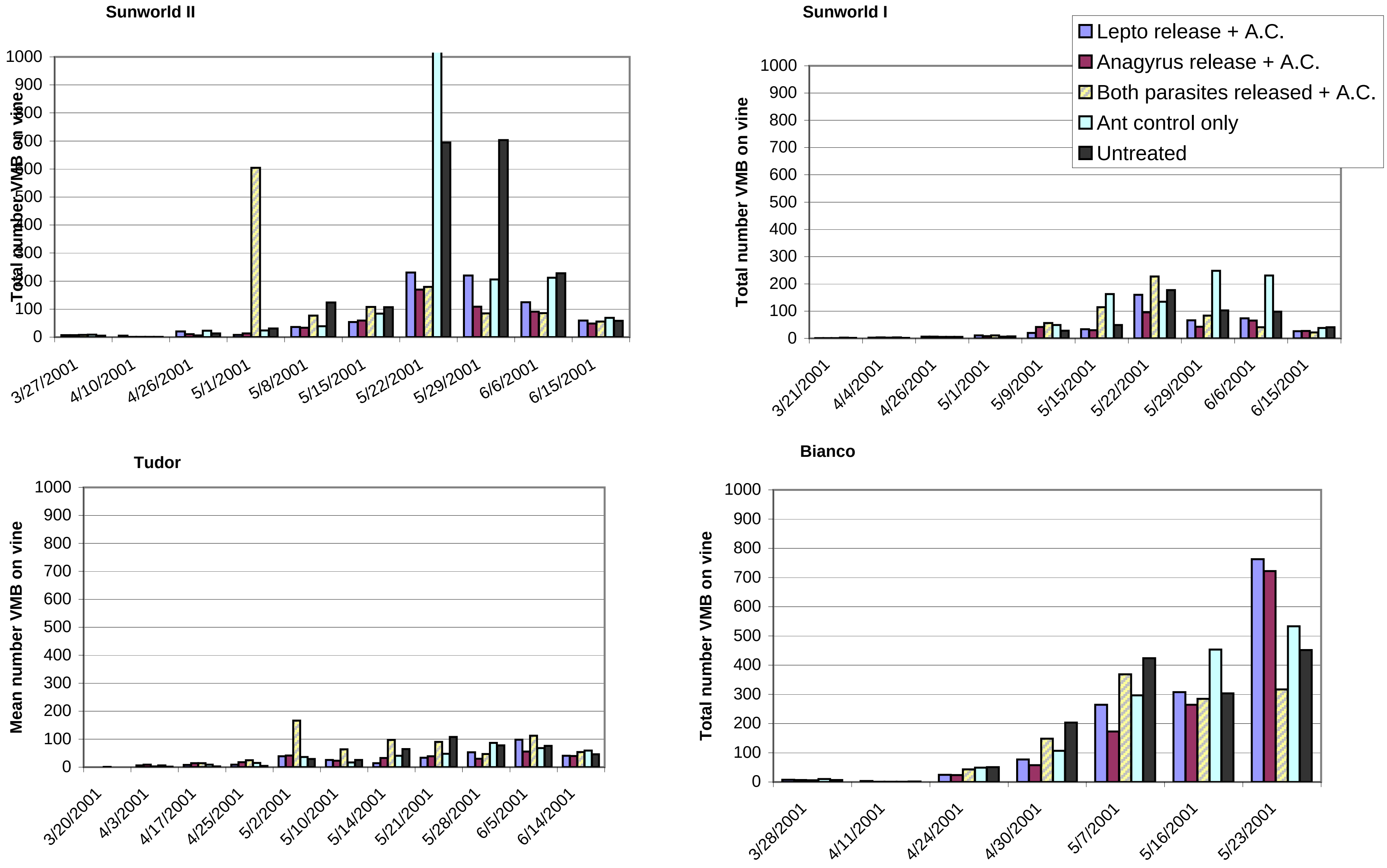


Figure 3. Mean number of Ants from four ranches, 2001

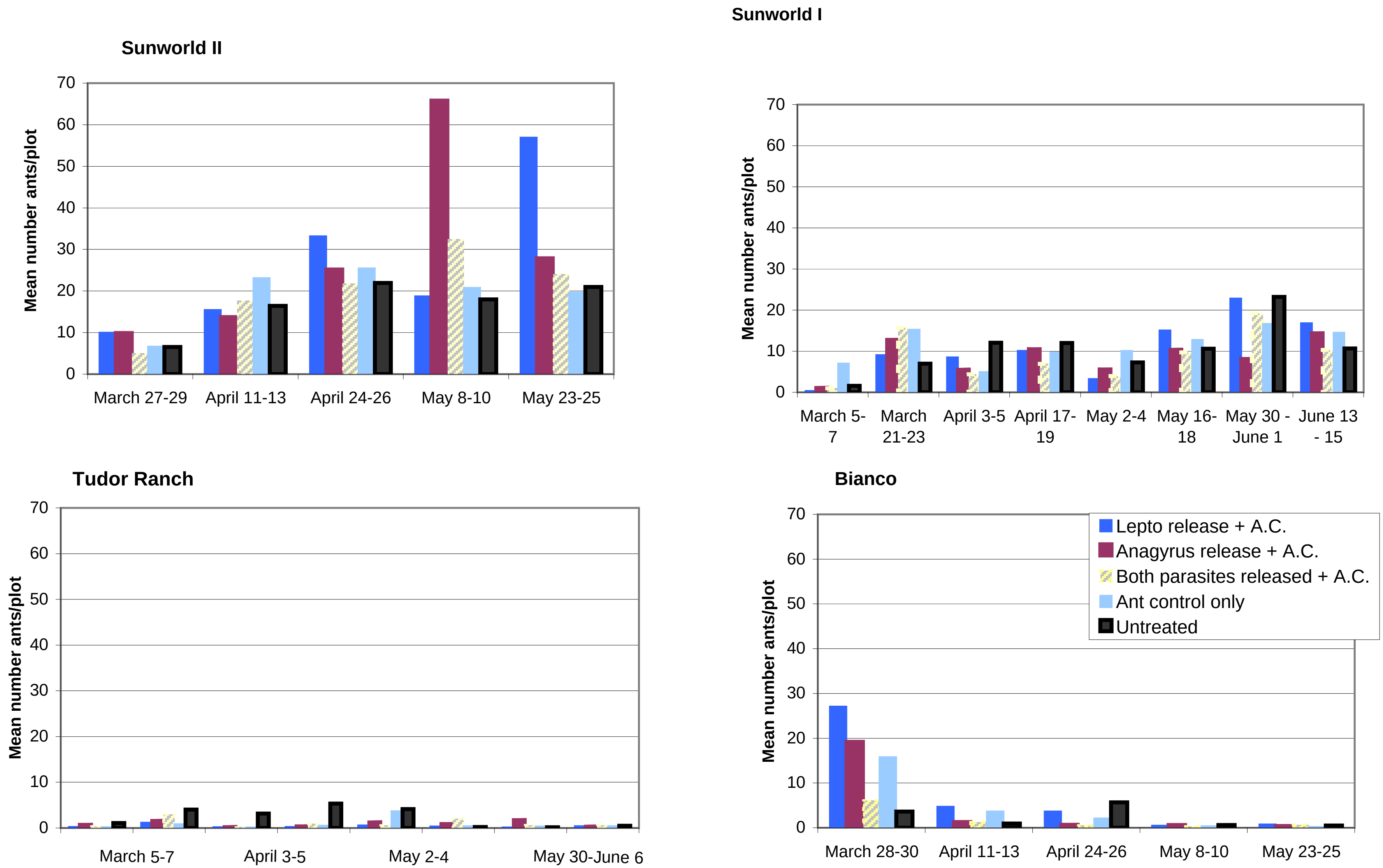


Figure 4. Mean # Anagyrus per Card 2001

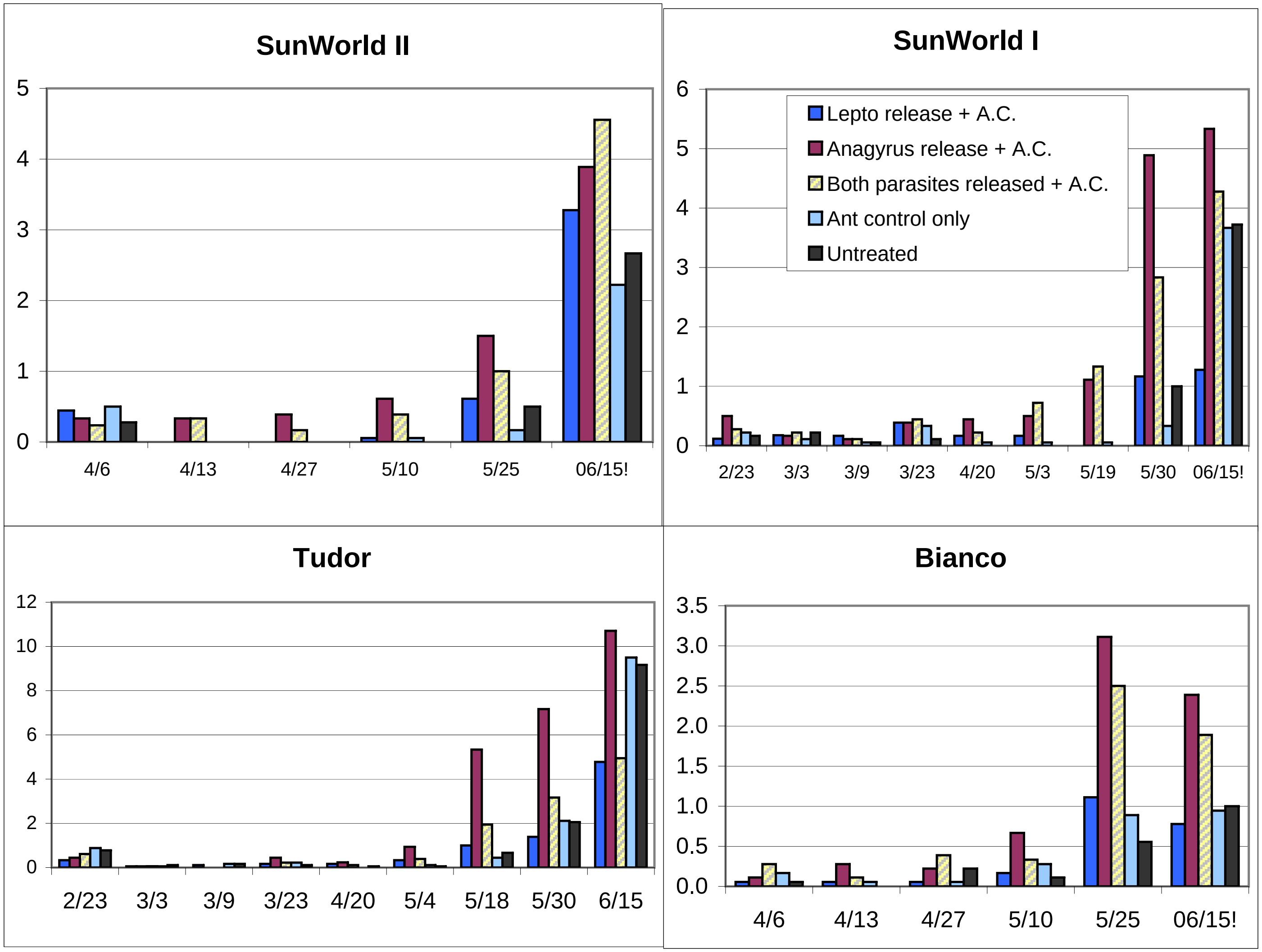


Figure 5. Mean # Leptomastidea per Card 2001

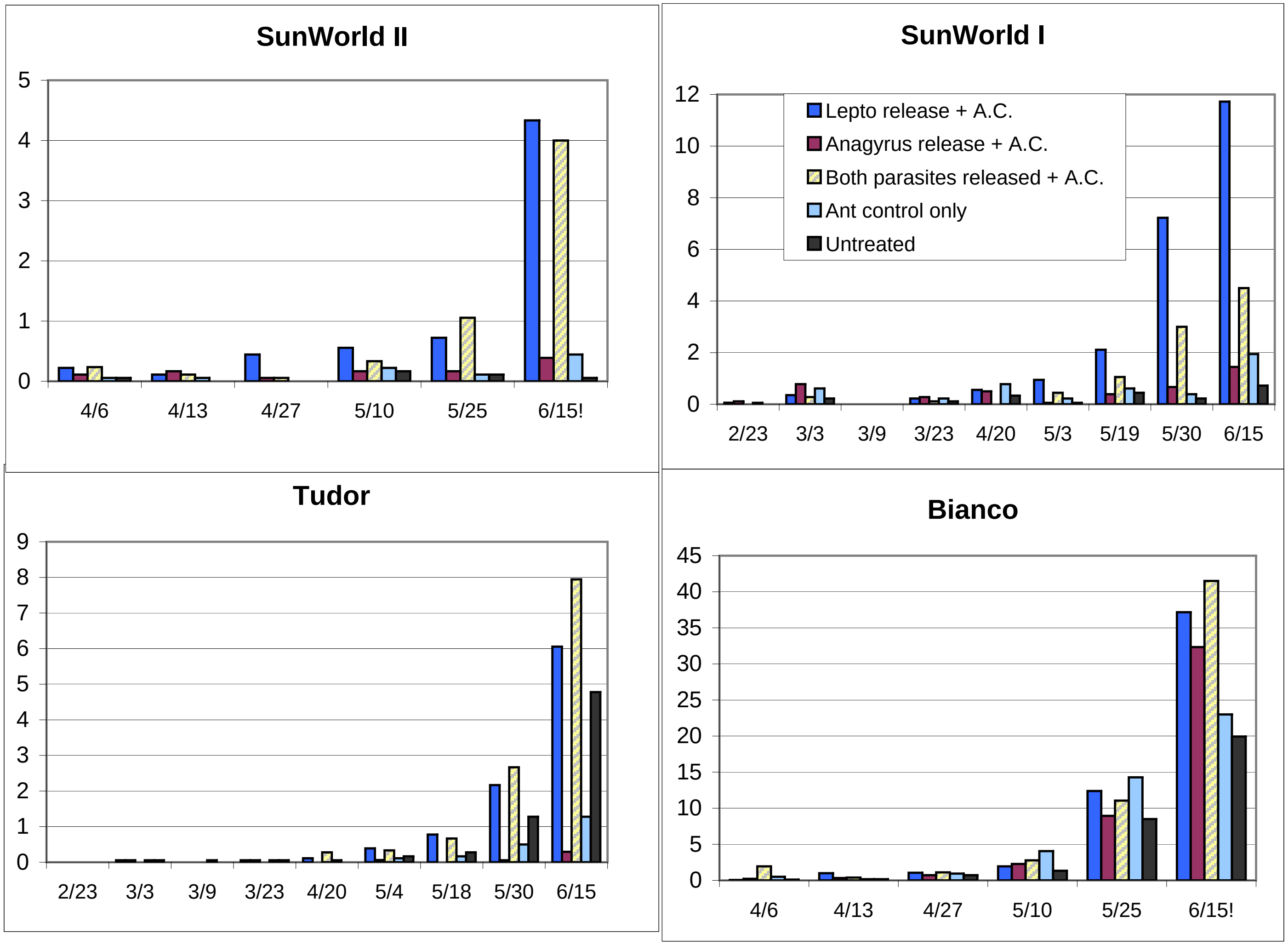


Figure 6. Adult Parasite Emergence by Field in Each of Four Treatments

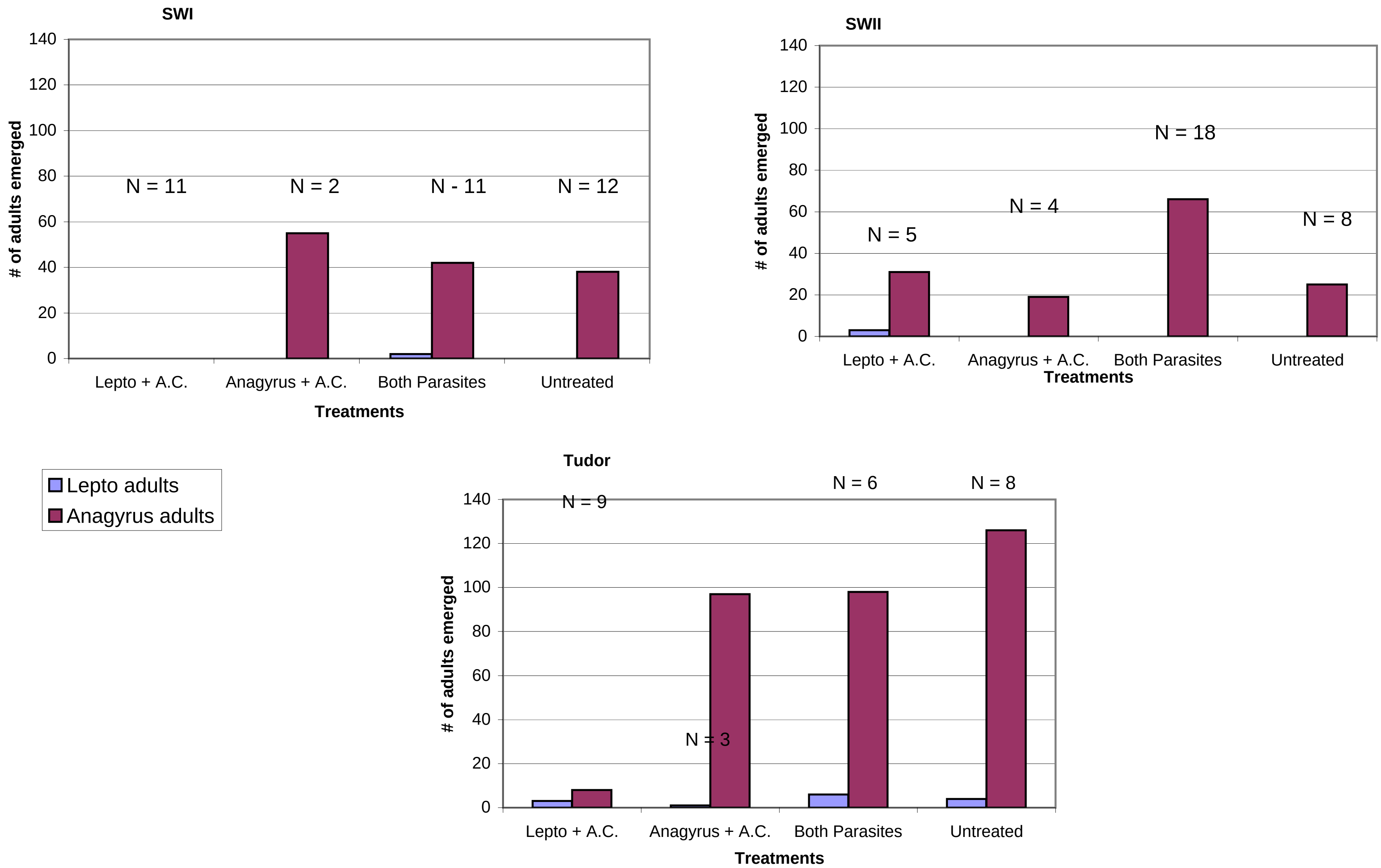


Figure 7. Adult Parasite Emergence by Treatment in Each of Three Fields

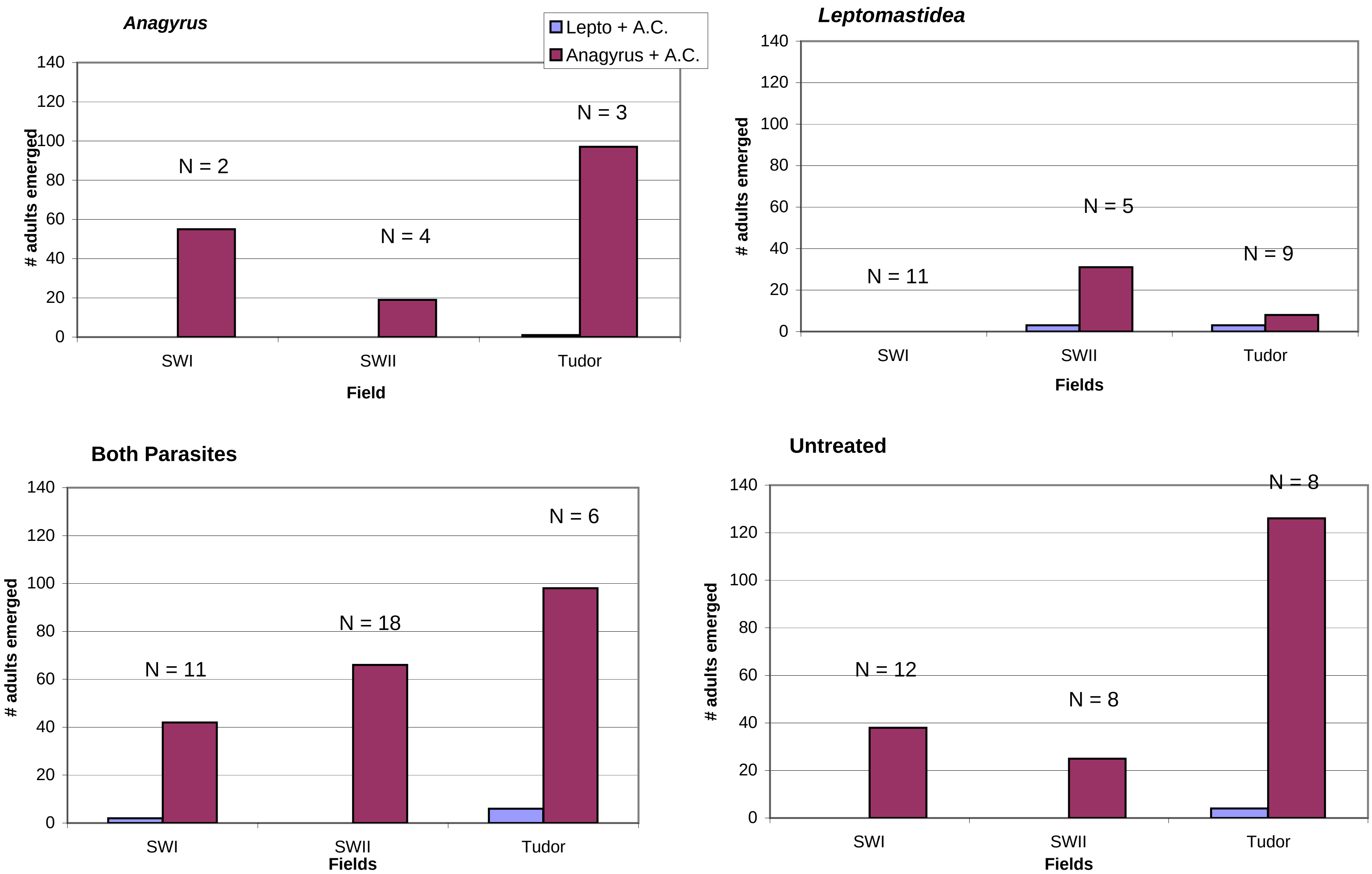


Figure 8. Damage from 4 Ranches, 2001

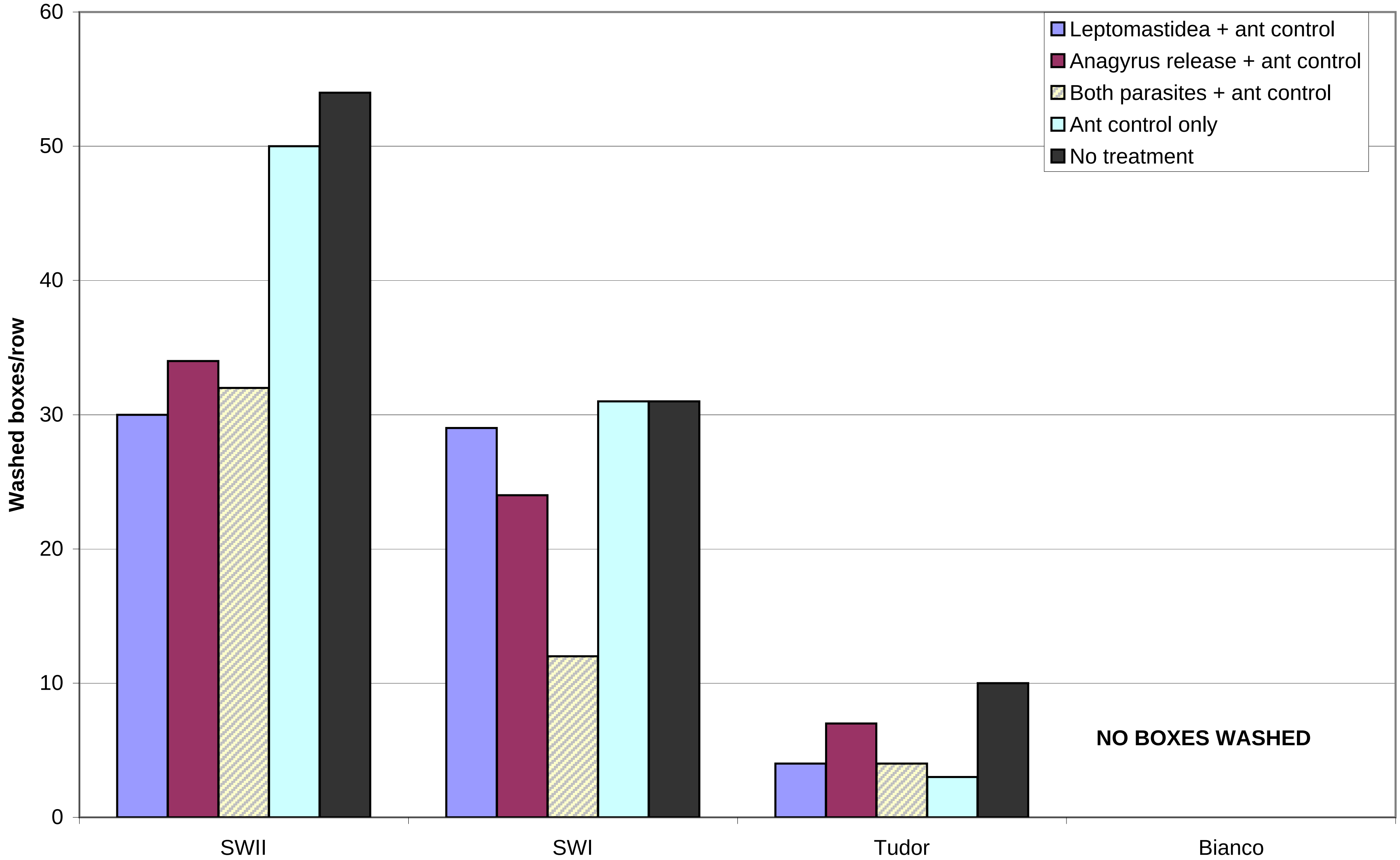


Figure 9. Yield from 4 Ranches, 2001

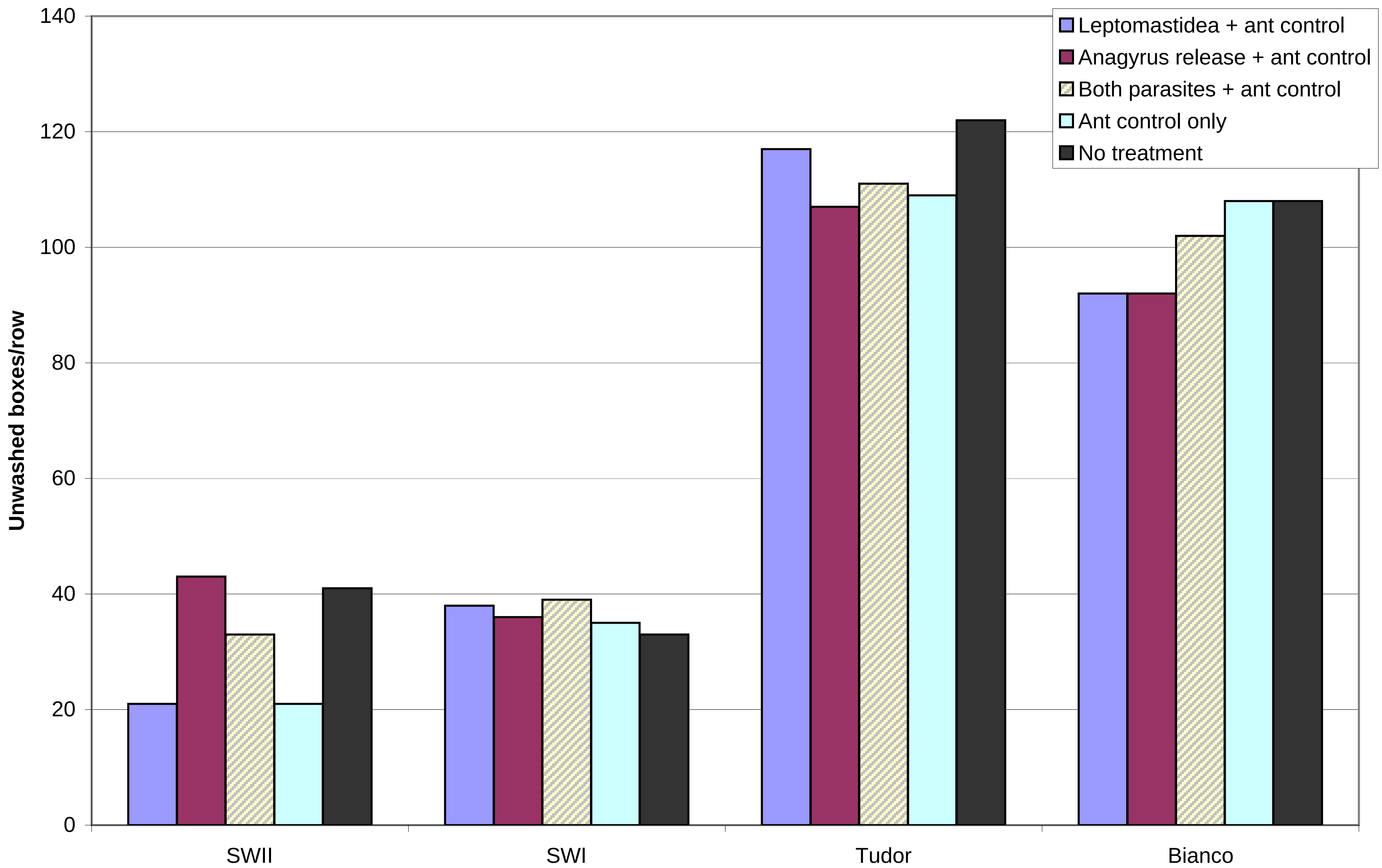


Figure 10. Coachella VMB – Summer 2001 New Experimental Design

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Y3	Y2	Y1	N1	N2
12,000/ROW	**8,000/ROW**	**4,000/ROW**	**NO PARASITE RELEASES**	**NO PARASITE RELEASES**
6000 ANAGYRUS/CUP MIN. 2 CUPS/ROW	4000 ANAGYRUS PER CUP MIN. 2 CUPS PER ROW	2000 ANAGYRUS PER CUP MIN 2 CUPS PER ROW		
HIGH # PARASITE RELEASE	**MED. # PARASITE RELEASE**	**LOW # PARASITE RELEASE**	**ANT CONTROL ONLY**	**NO TREATMENT**
YELLOW	**WHITE**	**RED**	**BLUE**	**ORANGE**

N
(SWI &
TUDOR

W
(SWII)

CHANGE IN TREATMENTS: NONE IN N1, N2

NEW TREATMENTS

LOW: Y1 = 2000 ANAGYRUS/CUP: (MIN) **2 CUPS/ROW** = **4000/ROW**

MED: Y2 = 4000 ANAGYRUS/CUP: (MIN) **2 CUPS/ROW** = **8000/ROW**

HIGH: Y3 = 6000 ANAGYRUS/CUP: (MIN) **2 CUPS/ROW = 12,000/ROW**
NEED MIN 18 CUPS EACH WEEK FOR 3 FIELDS (6 CUPS/FIELD)